EXPERIMENTAL INFECTION WITH THE VIRULENT, CENTRAL-EUROPEAN, MURINE LEPTOSPIRA POMONA STRAIN IN THE PIG

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Abstract. The virulent, murine Leptospira pomona strain isolated from Apodemus agrarius was used in an experimental infection of six pigs aged 4—5 months. The clinical course of the infection was inapparent, both the blood picture and the uptake of food were normal. All infected pigs produced antibodies against L. pomona at titres from 1 : 3 200 to 1 : 50 000. The reisolation of leptospires from the blood of the infected pigs was successful in one case only, and that on day two p.i. Throughout the course of our experiment, no microscopic evidence was obtained of the presence of leptospires in the blood of the infected animals. Of the six guinea pigs injected repeatedly with the urine of the infected pigs, antibodies against L. pomona were detected in two of these at titres 1 : 3 200 and 1 : 8 400. However, no direct proof was obtained of leptospires in their kidneys. Leptospires were isolated from the kidneys of two of the infected pigs, at days 10 and 21 p.i. respectively. As suggested by our results, the Central European, murine Leptospira pomona strain should be regarded as an independent biotype uncapable of causing a long-term leptospirosis and, hence, apparently unable to result in an epizooty in intensive pig husbandry. According to experimental evidence, Mus musculus can be a potential reservoir of the murine L. pomona biotype in Central Europe.

In Europe, where the serovar pomona is widely distributed (Kathrein and Mochmann 1987), it produces two types of leptospirosis foci (Mittermayer et al. 1961, Kmetý 1967), a natural focus with Apodemus agrarius as its main reservoir, and an anthropourge focus with the domestic pig as its main reservoir. It is the most important serovar in the etiology of leptospirosis of pig and cattle (Zwierzchowski 1967). In some countries, e.g., in the USSR (Lubaschenko 1962) and in the USA (U.S. Department of Agriculture 1954), it causes considerable losses to the breeds of domestic animals particularly cattle, pig and horse.

In the USSR, strains of leptospires of the serogroup Pomona, isolated from the common vole (Microtus arvalis), have been described as a new, independent subtype under the name Leptospira pomona madok (Semenova 1966). Later, this subtype has been placed in identity to the serovar pomona (Kmetý 1971, Borg—Petersen 1974), but Nicolescu and Moldoveanu (1974) continued to regard it as an independent subtype. Chernukha et al. (1974) obtained results of great interest and epizootiological importance in that they disclosed biological differences between strains isolated from small mammals and those isolated from domestic animals (L. pomona, L. "monja-

MATERIAL AND METHODS

We examined serologically six clinically healthy pigs aged 4—5 months with the microscopic agglutination-lysis test. We used these serovars and strains in the basic solution 1:100: 1. Icterohaemorrhagiae Fryšáva, 2. serovars Sorex Jahné, 3. canicola Canis 7, 4. and 5. pyrogenes
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**Table 1. The virulence of the strain L. pomona 7130/78 isolated on white laboratory mice.**

On January 31, 1979, 0.25 ml of the culture was administered to each mouse by an intraperitoneal injection.

**RESULTS**

One week before the experimental infection (February 14, 1979), all pigs were examined serologically with the 12 serovars of leptospires at the dilution 1:100 and, apart from these, with the three strains of L. pomona referred to earlier in the text, also at the titre 1:20. Pigs no. 1, 4, 5 and 6 reacted positively with L. tolverina monomorphae at the titre 1:800 and 1:100 respectively, pig no. 3 also with L. brasiliana (1:200). Before using the strain L. pomona 7130/78 in an experimental infection of the pigs, we tested its virulence on 4 white laboratory mice. Three days before the experiment, we examined the mice serologically, and their urine microscopically, three times, for the presence of leptospires. Bacteriologically negative was mouse no. 1 only, killed after one week p.i. Leptospires were reisolated from the kidneys of the remaining three mice at weeks 2, 3 and 4 p.i. respectively. All four mice were serologically positive with L. pomona 7130/78 at titres from 1:440 to 1:560. It was noteworthy that the titres of mice no. 2, 3 and 4 to the strain Simon differed greatly from those to strains Borg—Petersen, 7130/78 and Yub. 7130/77. The results remained unchanged in all three repetitions of the examination. By contrast, strains Yub. 32/II/77 and 7130/78 isolated from A. agrarius, reacted in the same titres. The results of the experiment are surveyed in Table 1.

In addition to confirming the virulence of the strain, the experiment made with mice showed that the infection with the murine biovar of the Central European L. pomona strains caused a prolonged leptospirosis in the house mouse (Mus musculus) that persisted for one month at the minimum. This finding provided reliable evidence that the species might be capable of acting as an important, potential reservoir.

We infected pigs no. 3, 4, 5 and 6 with the strain 7130/78 on February 19, 1979: pigs no. 3 and 5 perorally (5 ml of the culture in one liter phosphate buffer pH 7.2 administered as a drink), pigs no. 4 and 6 by injecting intramuscularly 2.5 ml of the culture. Pigs no. 1 and 2 served as controls. Owing to negative results, we infected the controls, pigs no. 1 and 2, on April 23, 1979 by injecting intraperitoneally 2.5 ml of the mentioned culture of leptospires. Table 2 surveys the results of repeated, serological examinations of the pigs. All six pigs reacted to the infection with the production of antibodies against L. pomona. It was of interest that pig no. 1 reacted as early as on day 4 p.i. to L. pomona Borg—Petersen at a titre of 1:600. The titres attained their peak at weeks 2 and 3 p.i. respectively, i.e., 1:50,000 (pigs no. 2, 3, 4), 1:6400 (pig no. 6) and 1:3200 (pig no. 5). In pig no. 5 killed at week 271
21 p.i., it was still possible to detect titres against *L. pomona*, of relatively low values (1:100, 1:200 and 1:800).

Throughout our experiment, there were no apparent clinical signs of the infection, the temperature, the blood picture and food intakes remained normal. We attempted to reisolate leptospires from the blood of the experimental pigs on days 2 and 4 p.i., but succeeded in one instance only (pig no. 1) on day 2 p.i. From the second week p.i., we tried without success to detect leptospires in the urine by means of a dark-field examination. Similarly, we failed to recover leptospires from the kidneys of guinea pigs injected repeatedly with the urine of infected pigs; three of the animals were negative even serologically. One guinea pig injected with the urine of pig no. 1 responded positively to *L. pomona* (strain Simon) at the titre 1:1 600, strain Borg-Petersen 1:3 200 and strain 7130/78 at 1:3 200; another guinea pig injected with the urine of pig no. 2 was positive at titres 1:1 600, 1:3 200 and 1:6 400; one guinea pig injected with the urine of pig no. 5 was positive at titres 1:200, 1:400 and 1:3 200.

Using the Korthof medium with 7.5% rabbit serum, we recovered leptospires from the kidneys of pig no. 1 and pig no. 2 killed at days 10 and 21 p.i. respectively.

**DISCUSSION**

As suggested by a comprehensive survey of literary data on an infection of the pig with *L. pomona* (Zwierzchowski 1967), clinical symptoms are indistinct particularly in the not quite young piglets. A temporary loss of appetite and an increase in the temperature are fairly frequent. On the other hand, leptospirosis becomes considerably prolonged (e.g., 122) and agglutination titres have been found to be present even after a longer period. Similar results were obtained by Shmatkova (1965) from an experimental infection of pigs. The author found neither clinical symptoms nor pathological changes in the organs; both leptospirosis and the serological positivity persisted for many months. Malakhov and Alekhin (1976) stated that the course of leptospirosis in the pigs was mostly asymptomatic and the rate of mortality low.

According to these authors, leptospirosis persisted in pigs with a spontaneous infection for as long as 13 months. The temperature was increased for a short period, from several hours to 1–3 days. The course of the infection was completely asymptomatic in two of six experimental pigs, their temperature, the blood picture and the food intakes, were normal. Apart from a prolonged leptospirosis, the course of our experimental infection with the murine *L. pomona* strain was similar to an infection with the pig biovar. While Morse et al. (cit. Zwierzchowski 1967) reisolated leptospires from pigs infected with the *L. pomona* pig biovar at as late as day 18 p.i., we succeeded in recovering them from one of our six experimental pigs infected with the murine *L. pomona* biovar, i.e., from the blood of pig no. 1, on day 2 p.i. However, there could not have been many leptospires in the blood of the pig, because we inoculated with it 4 test tubes but obtained a culture from one of these only. A fundamental difference was observed in the duration of leptospirosis in our experimental pigs as evident from the fact that we succeeded in reisolating leptospires from the kidneys of pigs that were killed on days 10 and 21 p.i. respectively. Owing to the small number of leptospires present in the kidneys of the two animals (pig no. 1 and pig no. 2), we failed to detect them in a dark-field examination of kidney preparations, and obtained a culture of leptospires from one of the four test tubes only inoculated with material from pig no. 1. The test tubes inoculated with material from pig no. 2, also contained at first very few leptospires. On the other hand, the kidneys of pigs infected with the *L. pomona* pig biovar, contain mostly large numbers of leptospires and, generally, these are released in masses. We failed to detect microscopically...
leptospires in the urine of our experimental pigs. In support of our results were those obtained from our experiment with the guinea pigs. In spite of a repeated infection, by way of injection, with the urine of the individual, experimental pigs, we failed to detect leptospires in their kidneys, and two guinea pigs only were serologically positive to L. pomona.

The postmortem examination of the six experimental pigs showed no pathological changes in their organs.

The results of our experiment confirmed the statement of the Soviet authors (Chernukha et al. 1974, 1975) that strains of L. pomona isolated from small mammals are host-specific. Also the Central European strains isolated from A. agrarius are an independent biovar adapted to A. agrarius as their main reservoir, and to other species of small rodents (A. uralensis, A. flavicollis, M. musculus) as their potential reservoir. Owing to the fact that the murine biovar is incapable of producing a long-term leptospiruria in the pig, its survival in pig breeds is most unlikely.

ЭКСПЕРИМЕНТАЛЬНОЕ ЗАРАЖЕНИЕ СВИНЕЙ ВИРУЛЕНТНЫМ СРЕДНЕЕВРОПЕЙСКИМ ШТАММОМ ЛЕПТОСПИРИА ПОМОНА

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Резюме. Вирулентным мышным штаммом Leptospira pomona, выделенным из Apodemus agrarius, экспериментально заражали 6 свиней в возрасте 4—5 месяцев. Заболеваемость была клинически заметна, температура, картина крови и прямые тесты были нормальны. После заражения у всех свиней образовывались антитела против L. pomona в титрах от 1:3 200 до 1: 50 000. Только в одном случае удалось реконструировать и кривую по Лептоспирозу, через 2 дня после заражения. В течение эксперимента L. pomona были обнаружены только в свиньях с антителами. Антитела против L. pomona были обнаружены только у 2 из них в титрах 1: 3 200 и 1: 6 400, но против остальных морских L. pomona не удалось. Лептоспироз свиней у животных, зараженных L. pomona, показывают, что среднезападные мышные штаммы L. pomona, в средней Европе может служить потенциальным резервуаром мышного биовара L. pomona.

REFERENCES


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